IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Atty Docket No. 106101-161)

In Re Application of:	Moshe Szyf	)	Group Art Unit: 1635
Serial Number:	08/652,425	)	Examiner: A. Wang
Filed:	May 30, 1996	)	
For:	INHIBITION OF DNA METHYLTRANSFERASE	)	

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Considered
7-10-03

Hon. Assistant Commissioner for Patents:

I, Jeffrey M. Besterman, Ph.D., hereby declare as follows:

1. I currently hold the position of Vice President at MethylGene Inc. ("MethylGene"). My professional experience, educational background, professional activities, and publications are detailed in the *curriculum vitae* which was attached as Exhibit A in my declaration of January 20, 1999, which was filed in the U.S. Patent and Trademark Office on February 24, 1999 together with a communication in connection with the above-referenced patent application (hereinafter the "Application").

2. I have personal knowledge of the invention disclosed and claimed in the Application.

3. MethylGene's efforts have been directed toward the reduction of the level of methylated cytosine in CpG dinucleotides to reverse the tumorigenic state of a cell and to reverse the growth of tumorigenic cells in human patients. This reduction in the level of methylated cytosine in CpG dinucleotides has been accomplished by a variety of ways, including a reduction in the level of DNA methyltransferase ("DNA MeTase") mRNA expression. As described in the Application, reduction of the level of methylated cytosine in CpG dinucleotides with a genetic-level inhibitor of DNA MeTase results in the reversal of the tumorigenic state of a cell. As illustrated in the following paragraphs, reduction of the level of DNA MeTase mRNA expression with a genetic-level inhibitor of DNA MeTase also appears to result in the inhibition of tumor growth and reduction in tumor mass in human patients.

4. The studies described herein were conducted under the supervision of the attending physician in accordance with Investigational New Drug ("IND") protocols authorized by myself and MethylGene.

5. The studies described herein are part of ongoing Phase I Clinical Trials approved by the United States Food and Drug Administration and the Therapeutics Products Programme IND submission program (Canada). The studies were performed in collaboration with clinicians at the following four sites in the United States and Canada: Johns Hopkins Oncology Center, Baltimore, Maryland; Princess Margaret Hospital, Toronto, Ontario; Ottawa Regional Cancer Center, Ottawa, Ontario; and British Columbia Cancer Agency, Vancouver, British Columbia.

6. The goals of the Phase I Clinical Trials, of which these studies described herein are a part, are determinations of safety and dose escalation for a genetic-level inhibitor of DNA MeTase. As further goals, these studies were designed to show that inhibition of the level of DNA MeTase mRNA and the inhibition of methylation of cytosine in a CpG dinucleotide in DNA using a genetic-level inhibitor of DNA MeTase (*e.g.*, an antisense oligonucleotide targeting DNA MeTase) results in the stabilization and/or reversal of tumorigenic growth in human cancer patients who were refractory to standard treatments for solid tumors, or who were untreatable.

7. MG98, a genetic level inhibitor of DNA MeTase, was used in these studies. MG98 is an antisense oligonucleotide that targets the 3' UTR of DNA methyltransferase mRNA at nucleotides 5218 to 2199, and has an IC_{50} value of 45 nM for inhibition of DNA MeTase mRNA. MG98 has the sequence 5'-UUC ATG TCA GCC AAG GCC AC-3,' and was chemically modified as follows: A equals 2'-deoxyriboadenosine; C equals 2'-deoxyribocytidine; G equals 2'-deoxyriboguanosine; T equals 2'-deoxyribothymidine; A equals riboadenosine; U equals uridine; C equals ribocytidine; and G equals riboguanosine. The underlined bases in the sequence of MG98 were 2'-methoxyribose substituted nucleotides, while the non-underlined bases were deoxyribose nucleosides. The backbone of MG98 consisted of a phosphorothioate linkage between adjoining nucleotides. MG98 was supplied as a lyophilized product containing 50 mg MG98 plus 40 mg lactose or 200 mg MG98 plus 160 mg lactose. The product was reconstituted in sterile 0.9% NaCl solution to achieve a final concentration of 25 mg MG98 per mL. MG98 was used for infusion promptly following reconstitution.

8. For infusions, a volume of physiological saline, equivalent to the volume of MG98 solution to be added, was removed from a 250 mL 0.9% NaCl injection USP

bag. An amount of MG98 solution necessary to achieve the targeted dose of MG98 per patient was then injected into the bag.

9. Dosage of MG98 was calculated in mg/m^2 of body surface area (BSA).

10. Patients were infused with the entire contents of a MG98-containing 250 mL USP bag for 2 hours twice weekly for three weeks (with no infusions every fourth week). Each four week period constituted one cycle of treatment. Peripheral blood mononuclear cells (PBMCs) were collected from each patient at various time points during treatment and the level of DNA MeTase mRNA in the PBMCs was quantitated.

11. The results of these studies demonstrated that treatment with two hour infusions of MG98 resulted in stabilization and/or reversal of tumor growth in some human cancer patients.

A. Patient UJ01 received two hour infusions of 80 mg/m^2 of MG98 twice a week for three weeks (with no infusions every fourth week) for six cycles of treatment.

As shown in Fig. 1, the level of DNA MeTase mRNA in patient UJ01's PMBCs decreased and remained diminished during the treatment time. Patient UJ01's cancer stabilized during the first 6 months of treatment. With continued treatment, patient UJ01's tumor mass has shown a reduction in size as determined by CT scan analysis.

B. Patient KO01 received two hour infusions of 40 mg/m^2 of MG98 twice a week for three weeks (with no infusions every fourth week) for seven cycles of treatment.

As shown in Fig. 2, after 14 weeks of treatment, the levels of MeTase mRNA in patient KO01's PBMCs decreased and remained low with continued treatment. More importantly, patient KO01's cancer has stabilized for at least 6 months.

12. In the studies described herein, no major toxicities have been seen thus far. Treatment was discontinued in patients where disease progression continued; while patients showing stabilization and/or reversal of disease progression are continuing to receive treatment.

13. To date, of the cancer patients who received treatment in the studies described herein, the patients who showed stabilization and/or reversal of disease progression had PBMCs which showed a reduction in the level of DNA MeTase mRNA. Thus, there was a positive correlation between an inhibition and/or reversal of tumor growth and a decrease in the level of DNA MeTase mRNA in PBMCs.

14. Given the results to date of the studies described herein, MethylGene is planning to enter Phase II Clinical Trials of MG98 for the treatment of cancer barring any unexpected limiting toxicities.

15. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed: Jeffrey M. Besterman
Jeffrey M. Besterman, Ph.D.

Dated: December 23, 1999

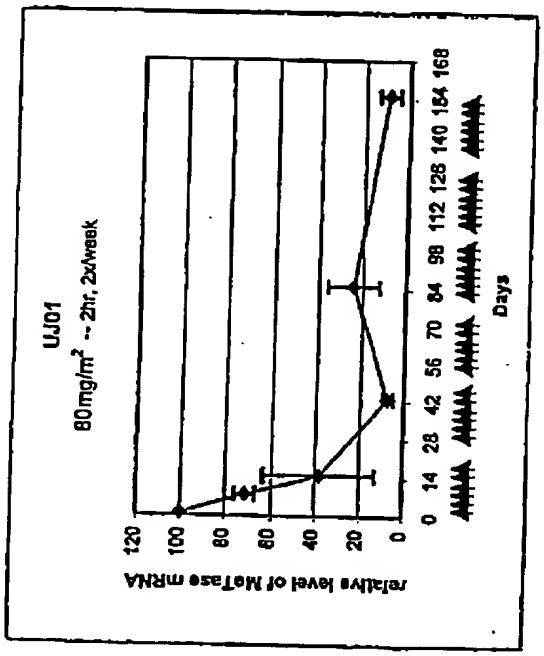

Figure 1

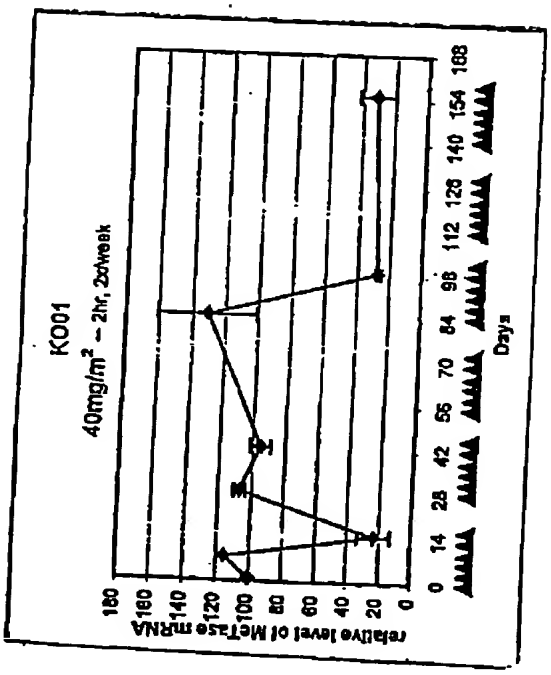

Figure 2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Atty Docket No. 106101-138

Serial No.:	08/481,876	)	
Filed:	7 June 1995	)	**Group Art Unit:** 1804
Inventors:	Szyf *et al.*	)	**Before the Examiner:**
Title:	Antisense Oligonucleotides Having Anti-tumorigenicity Activity	)	D. Curtis Hogue, Jr.

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Hon. Assistant Commissioner for Patents:

I, Dr. Moshe Szyf, hereby declare as follows:

1. I am a named inventor for the above-identified U.S. patent application. A copy of my *Curriculum Vitae* is appended to this declaration as Exhibit A.

2. The experiments described in paragraphs 3 and 4 below were conducted under my supervision and control.

3. Y1 or H446 cells were plated on a 6 well plate at a density of 80,000 cells/well. Antisense oligonucleotide phosphorothioates complementary to the DNA MTase coding sequence (about 0.5 to 20 micromolar) were added to the cells. The cells were similarly treated daily for 7 days. Then, the cells were harvested and 3,000 live cells were plated in soft agar, as described in Freedman and Shin, Cell 3: 355-359 (1974). Two weeks after plating, the number of colonies formed in soft agar were scored by visual examination. As shown in Exhibit B, attached hereto, a dose-dependent reduction in the number of colonies was observed for the antisense oligonucleotides.

4. Six to eight week old LAF-1 mice (Jackson Labs, Bar Harbor, ME) were injected subcutaneously in the flank area with 2 x 10^6 Y1 cells. Three days later, the mice were injected with 1-5 mg/kg antisense oligonucleotide phosphorothioates complementary to DNA MTase coding sequence. This dosing was repeated every two days. After one month, the mice were sacrificed and the tumor

size was determined. The antisense oligonucleotides caused a significant reduction in tumor weight and volume, relative to controls treated with a randomized or a reverse antisense sequence (See Exhibit C, panels A-D). In addition, the activity of DNA MTase enzyme was measured in the tumors and found to be significantly reduced relative to randomer treated controls (see Exhibit C, panels E and F).

5. The results discussed in paragraphs 3 and 4 above demonstrate that (1) antisense oligonucleotides complementary to the DNA MTase coding sequence are capable of inhibiting the ability of tumorigenic cells to form tumors and (2) these results hold up in generally accepted *in vivo* models for tumorigenicity. The soft agar plating approach discussed in paragraph 3 above has long been the method of choice for screening compounds for their ability to inhibit the tumorigenic potential of many types of cancer cells. The mouse model discussed in paragraph 4 above is the most widely used animal model for inhibition of tumor growth. Reference books on tumor biology have called the information provided by such animal models "critical to the development of cancer therapeutics" and referred to such animal models as "the best source of data for the study of tumor biology" (see Exhibit D at page 93). In fact, the subcutaneously transplanted tumor model, as described in paragraph 4 above, has been identified as being as informative as the long used mouse survival experiment (see Exhibit E). Consequently, the transplant syngeneic mouse tumor model has been widely used to test a wide variety of anti-tumor agents, as well as to study tumor biology, a fact that is well documented by many recent papers (see abstracts, Exhibit F). Cancer researchers would accept these experimental results as strong evidence that antisense oligonucleotides complementary to the DNA MTase coding sequence are capable of inhibiting tumorigenicity *in vivo*.

6. Recently, the scientific literature has come to express the view that inhibition of DNA MTase activity can inhibit tumorigenesis. A recent news article (Hopkin, The Journal of NIH Research 7: 26-28, 1995), quoted a researcher that "It was heretical to suggest that DNA methylation was important for initiating or causing progression of cancer. But it's becoming clear that this really is happening." The same article quoted another researcher's question of the ability of inhibition of DNA methylation to be directly translated to the treatment of human colon cancer on the basis that "The methyltransferase inhibitor 5-

aza-dC is too toxic for humans, and all cells require a certain level of DNA methylation to regulate normal gene expression. But if scientists could develop a drug that reduces methylation with less-toxic side effects, it would be a completely new way to treat cancer." (See Exhibit G). Another recent article (Laird *et al.*, Cell 81: 197-205, 1995) demonstrated that in a mouse heterozygous for the DNA MTase gene, further reduction of DNA MTase activity by the inhibitor 5-aza-dC reduced colon polyp formation from an average of 113 in a polyp-prone mouse expressing normal levels of DNA MTase to 2 in the 5-aza-dC treated heterozygote. (See Exhibit H). Thus, the premise of the above-identified patent application that antisense oligonucleotides complementary to the DNA MTase coding sequence can inhibit tumorigenicity has subsequently been supported by the scientific literature.

7. The ability of such antisense oligonucleotides to inhibit tumorigenesis is also supported by the absence of toxicity when these oligonucleotides are administered at concentrations which produce an anti-tumorigenic effect. In the experiment discussed in paragraph 4 above, no grossly observable toxic effects were found at an intraperitoneal injection concentration of 1-5 mg/kg. Moreover, in other experiments using phosphorothioate oligonucleotides in mice, no toxicity was observed at oligonucleotide concentrations up to 20 mg/kg. The above-identified patent application suggests an intravenous injection dosage of 1-5 mg oligonucleotide/ kg body weight (see Example 9, lines 12-14). In human safety trials, a 25-mer phosphorothioate oligonucleotide has been tolerated at dosages of up to 3.2 mg/kg oligonucleotide, with further increasing doses expected to be administered. Thus, antisense oligonucleotides complementary to the DNA MTase coding sequence provide effective inhibition of DNA MTase expression *in vivo*, without the toxicity associated with 5-aza-dC.

8. It is a routine matter to identify antisense oligonucleotides complementary to the DNA MTase gene which have antisense activity. For example, in our studies, we have tested 8 oligonucleotides complementary to the coding sequence for DNA MTase and have found that 4 of these were active as inhibitors of DNA MTase expression. Thus, a molecular biologist would only have to carry out limited and routine screening to identify which oligonucleotides complementary to the DNA MTase coding sequence will inhibit DNA MTase expression.

9. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed: _____

Dated: 4 November 1996